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Phytochrome
It Turns Plants On
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Groping for the Master Switch

Karl Norris was part of the Beltsville research team that succeeded in 1959—after an intensive quest—in detecting the plant pigment phytochrome, which serves as a biological light switch that controls flowering and other plant functions. In this month's Forum, he tells of the teamwork that made the discovery possible.

Norris went on to refine the spectrophotometer, an instrument now highly regarded in research and for many commercial applications. It operates on the principle that measuring the light passed through an apparently opaque object can reveal information about the object's unseen interior.

Now retired, Norris is a consultant for ARS and a collaborator with scientists at Johns Hopkins University developing a noninvasive device to monitor the content of oxygen in blood flowing to the brain.

In 1959, a diverse group of scientists at the Beltsville Agricultural Research Center carried out the first spectrophotometric measurement of phytochrome. Two of the key ingredients were the Pioneering Research Laboratories created by USDA in 1957.

These two labs, each created around an outstanding scientist, applied the concept of giving that scientist freedom to lead the lab into any research problem of interest.

One of the pioneering labs was built around Harry Borthwick, the other, around Sterling Hendricks. These two researchers, who had been collaborating for several years, could now work without the restrictions that so often creep into organizational structures.

A third key ingredient was the creation of the Instrumentation Research Laboratory within USDA's Agricultural Marketing Service and located in the Beltsville Agricultural Research Center's Building 002. I had the privilege of serving as the leader of the instrumentation lab, and I was able to convince my administrators that my research team should have freedom to work on projects not directly related to the lab's assigned mission.

A few convenient steps from 002 were buildings 006 and 007, which housed Hendricks and Borthwick. I became acquainted with Hendricks because he had a supply of lenses, mirrors, and other optical devices we could borrow for our projects. I subsequently hired Warren Butler, a biophysicist with a keen interest in plant physiology. He quickly became acquainted with Hendricks, Borthwick, and Bill Siegelman, who joined Borthwick's lab in 1957.

Without an assigned boss, this group became the team that found and measured phytochrome, succeeding where previous attempts to extract the pigment had failed. We had developed instrumentation to measure the optical properties of dense light-scattering samples, so we began a search for the elusive pigment using spectrophotometry of plant tissues.

Our initial attempts concentrated on fluorescence techniques. We did so because we knew the pigment had to be at a very low concentration, and we believed these techniques could best measure low concentrations.

Early in 1959, we abandoned this approach. We'd found that the fluorescence of chlorophyll and its derivatives masked any evidence of the photoperiod pigment. So we switched to trying to detect the change in absorption spectra that Hendricks had predicted in previous experiments on a wide range of plant material. We searched for plant material low in chlorophyll and other interfering pigments and high in the photoperiod pigment.

Siegelman and Hendricks had found a relationship between photoperiod response and anthocyanin formation, so we began to explore dark-grown seedlings that showed the presence of anthocyanin.

After several failures, one day Hendricks appeared in our laboratory with several dishes of dark-grown turnip seedlings for Butler and me to test. Working in the dark with a dim green light source, we packed plant tissue from the seedlings into the sample cell and measured the absorption spectrum.

Hendricks, a mountain climber, then took the sample cell, jumped up on the lab bench, and held the sample close to the fluorescent lamp to irradiate it with red light. Again we measured the absorption spectrum, and we could see that it had changed.

Next we irradiated the sample with a flashlight covered with several layers of colored film. The film blocked red light but transmitted far-red. Again we measured the absorption spectrum, with three pairs of eyes intently watching the pen on the recorder, each of us mentally trying to push the pen up as it passed the 660 nanometer region and down as it passed the 730 nm region. We could see a difference!

We irradiated again with red light and recorded the spectrum. To our delight, we had reversibility: red light decreased the absorption in the 660-nm region and increased the absorption in the 730-nm region. Far-red light did the opposite. We tested the sample several times and then called Siegelman so he could begin the extraction procedure.

The story of phytochrome detection illustrates one type of team research—an unstructured one. While this was the best way to solve the specific problem we faced, is it generally the best way to do research?

I think the answer must be no. Today and in the future, researchers will continue to rely on team approaches to solve many problems. From an administrative standpoint, however, organized teams are required. Still, administrators should avoid doing anything to discourage informal cooperation among researchers.

And we should always be alert to the possibility that an unorganized team—working together for the joy of finding new knowledge—can get the job done.—**Karl Norris**

Agricultural Research



Cover: Continuing phytochrome studies at the Beltsville (Maryland) Agricultural Research Center, plant physiologist Steve Britz measures horizontal light flux in a soybean canopy with a quantum sensor to assess the effects of shading.
Photo by Scott Bauer. (K-4197-7)



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Discovering Phytochrome

Tripping the Light Switch Fantastic

In 1918, a pair of U.S. Department of Agriculture scientists in northern Virginia asked two simple questions, launching an intellectual voyage still far from over. Their discoveries were the prelude to the search for what would prove to be one of nature's most important light switches. During the years 1936 to 1959, a dozen scientists at USDA's research center in Beltsville, Maryland, pursued and finally forced their quarry, phytochrome, into the daylight.

Today we know that phytochrome is a dual-form plant protein. It is switched back and forth by red light and by far-red, a zone at the horizon of our eyes' visual limits. By this transformation, phytochrome ordains whether a plant will start, or put off, making flowers. And in the next season, phytochrome wakens seeds to germinate or prolongs their sleep.

Skeptics, who reasonably believed phytochrome an illusion, dubbed it a "pigment of the imagination." Ironically, imagination was the key to unveiling phytochrome. It showed itself in clues the scientists saw in phytochrome's reflected reality—the measured life cycle of plants.

Balky Tobacco, Bullheaded Soybeans

The search began in 1918. Why, wondered botanist Harry A. Allard and physiologist Wightman W. Garner, did Maryland Mammoth tobacco not know when to stop making leaves and start making flowers and seeds?

The tobacco, first noticed in 1906, seemed a boon to growers. It grew as tall as 15 feet and put out nearly 100 leaves until frost would kill it. But what good was this oddity? It rarely flowered in Maryland, and it never produced seed in the field that could be used to plant the next crop. Its tantalizing ability to produce leaves seemed a mirage.

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In the 1940's, botanist Harry Borthwick compared different qualities of light on plants. A carbon-arc light salvaged from a Baltimore burlesque theater illuminates Biloxi soybeans.

And why, Garner and Allard puzzled, were soybean farmers being frustrated at spreading out their harvest time? They would plant the crop 2 weeks apart, but the plants would all set flowers at the same time.

The two researchers solved both mysteries with the same experiment. In retrospect, the test seems so simple it might have sprung from the naive fancy of a child—except no one had tried it before.

In July 1918, Allard and Garner grew some Biloxi soybeans and

Maryland Mammoth in pots. Some plants they left outside all day long. But every afternoon, they placed one group in a shed without windows, returning them outdoors the next morning.

The tobacco flowered 3 months earlier; the soybeans, 5 weeks earlier. Thus was born the concept of photoperiodism—an organism's response to the relative lengths of night and day.

At the time, the light/dark ratio lay mostly unexplored, an oddity in the standard explanation that plants grew and developed by laws of soil.



People didn't wait to learn what made photoperiodism work; they used it. Florists, no longer chained to a plant's outdoor season, began growing flowers indoors year round, serving each type the ration of light it needed to yield flowers. Plant breeders came out with crop varieties suited to day length, as fixed by the angle that a given latitude presented to the sun during a given season. Seed of Maryland Mammoth was successfully grown in Florida, where the plant flowered and set seed in the state's brief, mild days of winter.

It would take 41 years to isolate phytochrome—to get, in the words of Sterling B. Hendricks, “a bottle of the stuff” that was the trigger for photoperiodic response.

Night Lightening

In 1936, with little fanfare, USDA established a tiny research project on photoperiodism at its sprawling research center in Beltsville.

Botanist Harry A. Borthwick—heading the project—and physiologist Marion W. Parker were unsatisfied with using the conspicuous arrival of flowers as a meter of photoperiodism. Instead they examined “primordia.” These microscopic bodies emerge as the first visible signs of flowers.

They quickly discovered that only 2 “short” days—when nights were as long as 10-1/2 hours—made Biloxi soybeans produce primordia 5 days later.

climate, and the total amount of light they received.

The two USDA scientists soon found that different plants had different kinds of photoperiods. Maryland Mammoth and Biloxi soybeans, as well as chrysanthemums and others, flower in reply to the shortening days of late summer.

Lettuce, spinach, and the like are long-day—early summer—flowerers. And day-neutral plants like tomatoes and dandelions flower right up until frost.

Shortly thereafter, they found that this flowering could be prevented if a single, 30-second burst of light butted in on the long night. How could something so seemingly trivial—hardly enough light to wake a sleeping child—halt a plant's charge toward reproduction?

Again there was no answer, but breeders and horticulturists quickly enjoyed savings on light bills for their greenhouses. Instead of leaving the lights on for several hours each night to create a short night, they used a few minutes of light to get the same result.

At the time, the major focus of photoperiodism studies was to look for some kind of signal—perhaps a hormone—that travels from a plant's leaves to its fast-growing tips. But about 1940, Borthwick and Parker saw that they should home in on the leaf's interplay with light itself. About leaves, they knew much; they needed someone who knew about light.

The Disguise Takes on Color

They sought out Hendricks, a Beltsville colleague. An expert mountain climber, Linus Pauling's first grad student, and a brilliant chemist, Hendricks was one of the first Americans to use x-rays to study molecular structure.

The trio knew their quarry had to be a substance that could detect whether light was present. So it must be a pigment. But sunlight comes in a spectral stew of visible and invisible tints. Which color ingredient energized their

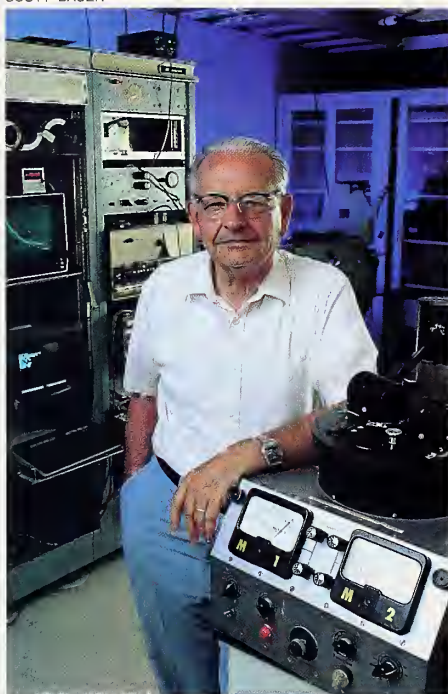


Botanist Harry Borthwick examines growth of cockleburs in this 1962 study of the effects of light on plants.

phantom pigment? And how could they find it?

Theoretically, the solution was simple. Just as water droplets carve the sun's rays into an arching rainbow, the scientists used a prism to filter light into its separate facets.

Hendricks knew how spectrographs worked and how to tinker with them. But most spectrographic work dealt with purified material, not the queer confederacy of solids and liquids that makes up leaves. Further, the light intensities used in spectrographs were small—designed for the needs of photographic film, not living plants. Finally, the spectra would have to be spread over a wide area; the researchers



Engineer Karl Norris displays the dual monochromator spectrophotometer which made history with its rapid and precise assays of phytochrome. (K-4201-7)

would gain nothing if several colors fell on the same leaf.

The solution was a \$50 experiment so elegant no large-scale research effort would ever have devised it. Their huge, 10-kilowatt carbon arc-light was “cadged from a Baltimore movie theater, with memories of pulchritude,” Hendricks reported later.

And somehow he obtained two large prisms, which were already historic. They’d been used by Samuel Pierpont Langley. Astronomer, physicist, and aeronautics pioneer, Langley had died in 1906.

For their test, light passed through the prisms to cast its spectrum 42 feet away, in a 7-foot swath, across 14 soybean plants at a time.

Phytochrome Sends the Signals—Sprout! Flower! Grow!

Your house may have photoelectric sensors that turn on your porch or walkway lights at dusk, then turn them off again at daybreak.

Much the same thing goes on inside each of the green plants in your yard—the grass, shrubs, and trees. Tiny photoreceptors—molecules called phytochrome—sense dark and light and send signals that turn on or turn off plants’ activities. Phytochrome’s signals, for example, can cause seeds to sprout or plants to flower.

And while any electrical engineer can tell you exactly how a photoelectric sensor works, today’s plant biologists can’t explain—at least not yet—exactly how phytochrome sends its signals to genes and other components that make up the molecular soup inside plant cells.

“We lack the finer detail,” admits Peter H. Quail, research director at the ARS/University of California Plant Gene Expression Center in Albany.

Knowing more details, he says, should “eventually give us unprec-

edented control over the green plants in our world.”

Tomorrow’s high-tech plants, for instance, might contain phytochrome-driven genes that confer traits such as improved flavor or higher nutritional value. Triggered by daylight, these supergenes would boost plants’ normal daytime production of flavor components or valuable nutrients.

To make these dreams and others come true, Quail’s team, one of six at the Center, works exclusively on phytochrome. The Albany researchers have already turned up several important new clues about this mysterious molecule.

Robert A. Sharrock, now at the University of Montana, has shown for the first time that at least three different genes, and probably five, direct a plant to make phytochrome. “We’ve always wondered,” says Quail, “how phytochrome could play a part in so many different events throughout a plant’s life. Now that we know there’s a family of phytochrome genes—and

that each gene possibly has a specialized function—phytochrome’s wide span of control makes sense.”

All genes contain components called promoters that turn genes on or off. To build new, efficient, and more powerful phytochrome genes for plants of the future, genetic engineers snip and prune promoter pieces, testing what’s left to see if they’ve deleted any important segments. Insiders call this work promoter bashing.

That’s how Wesley B. Bruce, now at Pioneer Hi-Bred International, Des Moines, Iowa, pinpointed three previously unidentified regions, or positive promoter elements, on a phytochrome gene from oats. He says two of the new elements seem to be interchangeable, but each requires a third element to work normally.

Bruce cut away pieces of the oat phytochrome promoter, then fused what was left onto a gene that triggers production of an easy-to-trace chemical.

Several groups of plants had been grown on 16-hour days to prevent flowering; each plant had been stripped of all but one leaf—the youngest mature one. When the test began, the scientists cut the plants' ordinary light exposure to 10 hours a day, which normally would induce flowering. But in the middle of the dark period, they turned on the spectral array for periods of 1 to 25 minutes.

After 6 days, they returned all the plants to long photoperiods. A week later they checked for primordia. The fewer the primordia, the greater the effect of specific light spectra on the pigment.

With this test, the pigment surrendered the first solid clue to its color. Since the plants responded most

strongly to red and yellow light, the pigment must be absorbing these hues. And that meant the active form of the pigment had to be blue or green.

This conclusion, obvious to any student of light, may not be clear to a lay reader. Any color that we see is made up of some combination of any or all of the red, yellow, green, and blue zones of the visible spectrum. Like a selective mirror, a colored substance reflects only what it cannot absorb. So something—like phytochrome—that absorbs only reds and yellows will reflect blue and green.

In 1948, tests with barley—a long-day species—proved that the same pigment governed flowering in long-as well as short-day plants.

This too was exciting, but a couple of years later the outlook seemed clouded. The spectrograph tests were important, but also awkward, imprecise, and time consuming.

Next to the spectrograph room was a doorway that would lead Hendricks, Borthwick, and Parker to faster progress.

Red and Far-Red Switchcraft

The door belonged to the Seeds Investigations Laboratory. There, the husband-and-wife team of Eben H. and Vivian K. Toole had for years been looking into why some seeds, like lettuce, need light to germinate.

Some 15 years earlier, physiologist Lewis H. Flint, another researcher in

He used a high-speed gene gun to shoot the material into healthy young rice seedlings and measured the amount of tracer chemical the little seedlings manufactured. High levels indicated, for example, that none of the missing promoter pieces were vital; low levels meant that Bruce had clipped off an important portion of the promoter.

Similar snooping into a rice phytochrome promoter revealed that the promoter probably can't turn on one of rice's phytochrome genes unless a special protein, GT-2, has attached itself to a specific portion of the promoter. Kato-yoon Dehesh at Albany, working with Wesley Bruce and Quail, discovered GT-2 in rice seedlings that had been bombarded with rebuilt rice promoters.

When plant geneticist Margaret Boylan at Albany first moved oat phytochrome—unmodified—into tomato plants, she ended up with dwarfed plants. They sported unusually dark-green leaves, normal-size fruit, and an excess of one type of oat phytochrome.

The research payoff for Boylan has been a new, fast way to see how plants engineered to produce carefully altered oat phytochrome differ from the first miniplants she created with the unaltered oat material.

Her experiments may not only yield new secrets about how phytochrome works, but may also provide tomorrow's consumers with a delightful surprise—tastier tomatoes.

The overabundance of oat phytochrome in tomatoes, she notes, increased tomatoes' color or pigmentation. She says other tomato studies show a correlation between high pigmentation, better flavor, and improved nutritional value.—By **Marcia Wood, ARS.**

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JACK DYKINGA



Geneticist Margaret Boylan inspects a tomato plant genetically engineered to include phytochrome from oats. The plants are dwarfed, but bear normal-size, highly pigmented fruit. (K-4028-6)

the lab, and E.D. McAlister of the Smithsonian Institution had set about finding that, indeed, germination was promoted most by red light.

Maybe that would be useful, Eben Toole mentioned to the photoperiodism group sometime in 1951 or 1952.

Would it ever! Experiments would take days instead of weeks. Moisten the seed for 16 hours, hit it with red light, then watch for germination in a few days.

Further, Flint and McAlister had seen something intriguing about far-red light and germination. Far-red light not only failed to promote germination; it stalled it. Hendricks, Borthwick, and Parker hadn't seen this—but it would also turn out to be true—in flowering. Discovering that far-red and red somehow foiled one another was critical in all further work to get a grip on the pigment.

On April 9, 1952, the loose-knit team of scientists came up with another magnificently simple find. Seed hit with red light germinated unless it was then hit with far-red; but if red again ensued, it *would* germinate. Incredibly, all that mattered was which color came last—even if the seed was struck by 100 alternating cycles of red and far-red.

That summer, the researchers confirmed the same switchability in flowering. Test plants flowered only if far-red light ended the sequence.

While fascinating, these discoveries gave the team no hard facts about the pigment's chemistry or its levels inside leaves. But they had reasoned this out: Their studies showed the pigment was probably blue or green and far more strongly colored than chlorophyll.

There was another clue: albino barley plants, while responsive to their light tests, showed no blue tinge. That meant the pigment's concentration was minuscule. To cause such dramatic effects on plants, the stuff had to be some kind of catalyst.

That, they surmised, meant it was an enzyme—and therefore a protein.

Still, though they could measure what the receptor protein did in plants, they couldn't detect it in a test tube. And virtually no one else was doing this kind of work; literature searches circled them back to what they had published themselves.

By 1953, Flint and Parker had left the team. But Borthwick, Hendricks, and the Tooles were soon joined by two physiologists, Albert A. Piringer and Robert J. Downs.

Then, around 1956, a third phase of informal collaboration with colleagues led the team to one final breakthrough.

A Bottle of the Stuff

Agricultural engineer Karl H. Norris [*See Forum, page 2*], who at the time headed up a USDA Agricultural Marketing Service lab, had worked for about 10 years on nondestructive ways to gauge the quality of produce.

To do this, he'd adapted several spectrophotometers so they would measure typical patterns of light absorption by goods such as eggs and apples. Ordinarily, these gadgets required material that was nearly transparent.

Joining Norris in 1956 was biophysicist Warren L. Butler; 1957 saw the final ingredient added, when Borthwick brought aboard Harold W. Siegelman, horticulturist turned biochemist.

That same year, the project received its first-ever operating budget, when Borthwick's lab was named one of ARS' two Pioneering Research Laboratories—the other being Hendricks' mineral nutrition lab.

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An early photo of Harry Allard in front of photoperiod houses at Arlington farms in Virginia. After being shut in these windowless sheds for part of each afternoon, tobacco and soybeans plants flowered weeks earlier.

The basic idea was to use the spectrophotometer to tally changes in light absorption by plant tissue that held the pigment protein. The researchers knew that red changed the protein into a form sensitive to far-red. So, that form (which turned out to be green pigmented) should absorb more far-red light than red. And vice versa: Far-red light should make the now red-sensitive (blue) form of the protein absorb more red than far-red.

For 2 more years, the team tried tissues from various plants: lettuce, cocklebur, albino barley, and the like. None worked.

In mid-June 1959, Hendricks showed up in Butler's lab with dark-grown turnip seedlings for the spectrophotometer. "To our amazement and delight, mixed with skepticism," Butler later reported, "we found that the difference spectrum between the red and far-red irradiated sample was precisely that predicted for phytochrome by the physiological action spectra" the scientists had been charting for years with Hendricks' large spectrograph.

Within 2 hours, Siegelman tried the technique with a sample of ground-up turnip seedling. It retained the red/far-red reversibility.

He then boiled a sample and tested it: no reversibility. This agreed with the prediction that the receptor was a protein, now destroyed by boiling.

Officially Named

The following April, the stuff in the bottle had its official name, a borrowed one combining the Greek words for *plant* and *color*. Butler suggested it half-jokingly, according to Borthwick. Phytochrome, which had once referred to all visible plant pigments, now named a dual-form protein—green in its red-sensitive form, blue when sensitive to far-red.

Later that summer, Hendricks was invited to speak in Montreal at the Ninth International Botanical Congress. He suggested Butler run a demonstration after his talk. Norris produced a portable photometer, rigged to a wall-clock-size meter.

In repeated trials in Beltsville, the meter's dial swung reliably between its 9-o'clock and 3-o'clock positions each time a sample was alternately beamed with red and far-red light.

With high confidence, Hendricks, Butler, and Siegelman set off by car for Montreal. Unfortunately, they did

"In spite of the failure, the audience appeared to be kind and accepting and even to believe that we probably had achieved what we claimed."—Warren L. Butler

not yet know that the far-red form of phytochrome is unstable once red light produces it in a seedling. This insight came too late to prevent their Montreal demonstration from becoming a dud.

Whenever the three scientists stopped for gas during the drive to Canada, someone would check the trunk to make sure the corn seedling samples were okay. But opening the trunk let in huge doses of red light, converting the red-absorbing form of the protein into the wobbly, far-red form. By the time they got to Montreal, the seedlings didn't hold enough phytochrome to nudge the photometer dial by one whit.

"However," Butler remembered later, "in spite of the failure, the audience appeared to be kind and accepting and even to believe that we probably had achieved what we claimed."

Since 1959, phytochrome has continued to resist scientific prying. Not until 1983 was a reliable means developed to purify the protein in a fully intact form.

It's still unclear where phytochrome resides in cells and exactly how it throws its genetic and behavioral switches in a plant.

It's not even absolutely settled whether the far-red-sensitive form of phytochrome is an enzymatic protein. A catalyst, yes, but not all catalysts are enzymes. Phytochrome, like some other plant proteins, may have a different way of filling its role as a biochemical amplifier.

In addition to phytochrome, scientists have uncovered two or three other classes of photoreceptors, such as those that absorb blue light and possibly even ultraviolet light.

Researchers today continue gathering and sifting clues that may open up secrets of phytochrome and other photoreceptors for human advantage. What may emerge could be new strategies to control weeds, to make better use of a crop plant's preference for certain shades of light, and to use biotechnologies to improve one plant by borrowing the genetic light switch from another.

Such stories cannot yet be written in full, but their outlines are being imagined.—By **Jim De Quattro**, ARS.

NOTE: The principal source of this account is "A Pigment of the Imagination," written by Harold McGee and published by ARS in 1987 for the dedication of the ARS Plant Gene Expression Center in Albany, California, a cooperative effort of ARS and the University of California, Berkeley. ♦

Dress-for-Success Mulch

RICHARD NOWITZ



In an experimental tomato patch, mulches of various colors are checked for their effects on plant growth, yield, and insect populations. (K-4104-11)

Tomatoes are partial to red, potatoes favor pale blue or white, and turnips don't think orange is too bad.

Growing tomatoes over red rather than conventional black plastic mulch increased harvests of number-one-quality fruit by 10 to 15 percent, according to plant physiologist Michael J. Kasperbauer and soil scientist Patrick G. Hunt, both with ARS' Soil and Water Conservation Research Center in Florence, South Carolina.

Pale blue or white mulch increased the harvest from potatoes by as much as 15 percent. It's not the colors of the mulch that enhance the harvests, but

the differences the colors make in the light reflected onto the plants.

The colors can also have an impact on taste and protein levels in leaves and might even provide protection from insects in some cases.

Mulch itself is commonly used by farmers and gardeners. Soil coverings such as plastic and straw conserve soil moisture and keep weeds down. American vegetable growers use 200 million pounds of plastic mulch a year.

In the spring, heat-absorbing black plastic mulch is used to warm the soil and give plants a head start. In southern areas, white plastic is used to reflect light and reduce soil

temperatures to help late season crop production.

But it isn't the temperature, moisture, or weed control benefits that interest Kasperbauer and Hunt. Mulch is simply a convenient vehicle to let them place colored light reflectors below a field-grown crop without interfering with the incoming light.

"Using black mulch is known to be good for plants, but we've found using the right color is super good," Hunt says.

Light, including sunlight, is made up of various amounts of different wavelengths. Different combinations of wavelengths show up as different colors, as can be seen when sunlight is

broken into the colors of the rainbow. So-called white light is actually a blend of many colors of light.

When light reaches a colored surface, some wavelengths are absorbed and others are reflected, altering the color of the light reflected from surface.

"Plants are extremely sensitive to the color of light," Kasperbauer says. "They are particularly sensitive to the blue, red, and far-red portions of the light (color) spectrum."

Far-red, also called near infrared, is just beyond the spectrum visible to the human eye.

Light that has a low far-red-to-red (FR/R) ratio will cause a plant to develop a shorter stem and bigger roots. A higher FR/R ratio causes a plant to direct more new growth into the shoots, resulting in a taller plant with more leaves.

"These responses are understandable when you realize each green leaf reflects an increment of far-red. So a plant with many neighbors will get more reflected far-red and a higher FR/R ratio," Kasperbauer says. "A plant responds to the light signal that says that it has many neighbors by growing taller than its neighbor. With a low FR/R ratio, a plant senses no competition, develops more branches, and sends more nutrients to the roots."

What color mulch is most effective depends on what a grower wants.

"Obviously, if you're interested in a crop like turnips or potatoes, you want to increase growth below the ground; if you are growing tomatoes, more fruit on the plant is what's desirable," Kasperbauer says.

Kasperbauer began his work in photobiology in 1961 with a team of ARS scientists at the Beltsville Agricultural Research Center that pioneered the research on a plant pigment called phytochrome.

Phytochrome, which was discovered in a project headed by Harry A. Borthwick and Sterling B. Hendricks,

is considered to be the universal regulator in plants. Plants sense the quality of surrounding light through chemical reactions in phytochrome.

Phytochrome exists inside plants in two interconvertible forms. One form absorbs only red light, which causes it to undergo a chemical transformation and become the other form. The other form will absorb only far-red, and then it becomes the other, red absorbing only, form. The ratio between the two forms in the plant depends on the ratio

The colors can also have an impact on taste and protein levels in leaves and might even provide protection from insects in some cases.

of far-red to red light and regulates the use of resources within the plant.

In one of his experiments in the late 1960's, Kasperbauer measured the amount of visible and far-red light in sun flecks that reached the soil surface in a field of tobacco and in those on a plant-free road surface near the field, but away from any plants. Sun flecks are bright concentrated spots of light reflected from a surface.

He found those close to growing plants had more far-red than sun flecks on the road surface. The amount of far-red was also higher where plants were closer together.

"The denser the plant population, the higher the FR/R ratio was and the more it told plants to grow taller to adapt to the competition," says Kasperbauer.

During the late 1970's in Florence, Hunt was investigating the effect of environmental stress, particularly water

stress, on different strains of nitrogen-fixing bacteria in soybeans.

Hunt found an odd response that could not be attributed to changes in water availability.

"Without irrigation, we found a 10-percent increase in the yield of soybeans that were planted in rows that ran east to west compared to north-south rows," Hunt says.

"However, with irrigation and no water stress, we got the highest yields in the north-south rows," he adds.

When Kasperbauer joined the Florence lab in 1983, he and Hunt reasoned the row direction might be affecting the FR/R ratio reflected on the plant canopy.

When they examined the light in the field, they found that light falling on and reflected from east-west rows consistently had a little less far-red in it.

Less far-red means more growth in the roots and therefore more nodules for nitrogen-fixing bacteria and drought tolerance. More far-red produces more shoot growth and is most useful for soils with high nitrogen levels and plentiful rainfall or irrigation.

"While the difference isn't much, you're getting an increase for just turning a tractor around—maybe only a bushel or two an acre, but at more than \$5 a bushel, that's a nice increase," Hunt says.

Subsequent experiments showed the sun-tracking ability of leaves increased the amount of far-red a plant in a north-south row was exposed to in the course of a day. "Plants in north-south rows could track the sun all day. They got a slightly higher FR/R ratio, especially near the end of the day when the leaves faced the sun and reflected far-red back onto the next row of soybeans," Kasperbauer says.

"Since we already knew plants responded to the FR/R ratio in a controlled environment and to reflected far-red from competing plants, the next step was to look at

plant responses to light from other surfaces," Kasperbauer says.

They raised questions about how different soils, which come in a range of colors from almost white sand to the black soil of the Midwest, reflect sunlight. They also questioned if the presence of crop residues in a field represented a "soil color" change and caused changes in the light reflection.

The two researchers began testing the influence of reflected light on plant growth with boxes of different colored soils from different locations. But they also had to control the soil temperature that is influenced by the color of the soil, since it also affects plant growth.

To isolate the color reflectance effect, they used Styrofoam panels, each covered with a layer of a different colored soil. There were obvious differences in plant growth over different soil colors, even when the insulation panels kept the temperatures the same.

During the following summer, the scientists found painted styrofoam panels gave the same plant growth responses as the soil-covered panels, if they reflected the same wavelengths and wavelength ratios.

Enter Colored Plastic

In 1985, D.R. Decoteau, a Clemson University horticulturist who was working on tomato productivity, began working with Hunt and Kasperbauer.

Since plastic mulch is widely used for tomato cultivation but available only in black or white, the scientists had to paint plastic in order to create other colors.

Paint from the local store was used to convert black mulch into the other colors. Each paint had to be measured with a spectrophotometer to record the wavelengths (colors) of the light reflected off of it.

The magnitude of impact that colored mulch had on the first tomato experiment surprised them, Hunt

RICHARD NOWITZ



Wavelength of light affects plants both above ground and below. Plant physiologist Michael Kasperbauer (left) and Clemson University professor Bruce Fortnum examine the influence of light on cotton shoots and nematodes that infect their roots. (K-4102-11)

admits. They expected differences in growth, but red mulch caused a 20-percent increase the first year in number-one fruit—the ones that bring the most money—that was amazing.

Using red mulch treatment didn't always give as much as a 20-percent increase. The second year, the difference was 37,057 pounds of fruit with red mulch compared to 32,921 for those mulched with black plastic, a difference of about 12 percent. "That's a pretty good return for a change of surface color," Hunt points out.

Since that first year, the scientists have tested red, orange, yellow, blue, green, white, aluminum, black, and various combinations of these colors on crops such as peppers, cotton, soybeans, southern peas, turnips, and potatoes as well as tomatoes. Turnips were used because both the greens and roots are used as food.

For all crops, the key is the amount of reflected far-red and FR/R ratio. "It is not as simple as taking the first bucket of red paint or roll of red plastic off the shelf," Kasperbauer says. "The amount of far-red reflectance needs to be verified with a spectrophotometer. Plants are even more discriminating between colors than the human eye is."

One spring, when the spectrophotometer was out of service, the researchers mixed two batches of blue paint by eye.

"To our eyes, the two batches looked exactly the same, but the results at the end of the season were different," Kasperbauer says.

When they ran spectrophotometric measurements on the two blues, they were very different in the amount of far-red and the FR/R ratio.

"They looked the same to our eyes, but the spectrophotometer didn't see them that way and neither did the plants," Kasperbauer says.

When colored mulch begins to be produced commercially, the production of each color will have to be very precise. There are many mixtures of color that appear red to the human eye, but it takes a particular red to stimulate a tomato plant correctly. "Not just any red is going to give you more and better tomatoes," Hunt says.

Colored mulch will benefit the home gardener as much as the commercial producer, according to Hunt and Kasperbauer.

A patent application has been filed for the colored mulch approach. ARS is seeking a company with which to sign a cooperative agreement to develop color concentrates that reflect the proper wavelength combinations for several crops and eventually produce a commercial product.

In the commercial product, different colors might be used to enhance different stages of a plant's growth, Hunt says.

"Maybe we can layer colors of mulch so as one degrades, it reveals another



Red, brown, white, and tan soil reflect different wavelengths of light that affect the growth of cotton seedlings. Soil scientist Patrick Hunt (left) and plant physiologist Michael Kasperbauer compare results. (K-4105-6)

color underneath because you might want a color to stimulate rapid root growth around seedlings transplanted into a field and later a colored mulch to increase shoot growth," Hunt says.

Patterns of color are also a possibility. One color could intensify one part of the sunlight, while another color enhances another part of the light. "If you flew over fields of mulch like that, it might look plaid or checkered," Hunt says.

Early on, Hunt tried a combination of blue and fluorescent orange checkers. Fluorescent colors reflect light that is very high in red and very low in far-red.

"From 100 yards away, the light reflected from the fluorescent orange made the plants look like they were on fire," Hunt says.

The combination didn't work very well on potatoes, although it had some beneficial effect on turnips.

"Actually, a few people here think I started with orange just because I grad-

uated from Clemson University with its traditional orange tiger paw," Hunt says.

Turnip studies turned up another possible effect of the colored mulch.

"It seems that the color of the mulch had an effect on the taste of the turnips," Hunt says.

They used turnips for the preliminary taste test because both the root and the greens are food crops and a change might occur in either part.

Blue mulch, with less far-red, appears to cause a greater accumulation of the compounds responsible for turnip flavor.

"Both the turnip and the greens in that case had a more bitey, less sweet taste," Hunt says. "Of course, the taste tests have been very informal at this point and much more work would need to be done to track any taste effects.

Another possible benefit of a custom-colored mulch: repelling insect pests. With the help of entomologist Steven Roach, the researchers recently completed a 2-year study that looked at

insects on cotton grown over seven colors of mulch: red, yellow, green, blue, black, white, and aluminum silver, as well as bare ground.

Each week, the cotton plants were vacuumed to collect and count the insects living on the plants.

Preliminary results showed the color of the mulch has some impact on the number of insects found on the cotton plants, although not all the data have as yet been analyzed.

While a few aspects of the commercial use of colored mulch remain to be fine-tuned, "this may be one of the first major field applications to come from the scientific discovery of phytochrome," Kasperbauer says. "Colored mulch is certainly based on solid photobiology."—By **J. Kim Kaplan, ARS.**

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Erosion Can't Hide From Laser Scanner

Particles of topsoil blown by wind will bounce along the soil surface and finally escape a field, leaving it less able to support crops. Water will wash away valuable topsoil and nutrients. And how rough the soil surface is influences whether the soil will erode.

Until now, soil scientists have had no suitable technique to measure soil roughness—or microtopography—on the small scale.

ARS soil scientists Joe M. Bradford and Chi-hua Huang, of the National Soil Erosion Research Laboratory in West Lafayette, Indiana, have developed a portable scanner that can. It measures the tiny ridges left in the soil by tilling or clods of soil particles that clump together naturally.

What does the scanner do? It measures soil elevation by shining a low-power laser beam onto the surface and detecting the position of the laser spot reflected from the soil with a 35-mm camera. In place of film, the scanner camera uses electronic circuitry somewhat similar to that in a video camera to transmit the spot's position to a small computer about 30,000 times a minute.

The laser and camera are mounted on the frame of a motor-driven carriage. The computer controls the carriage movement.

At the end of a scan, a microtopographic map is stored in the computer. Scientists can analyze it immediately and can compare it to previous maps to see whether erosion has occurred.

Before the scanner, researchers used to lower a single pin or row of pins onto the soil surface and register the pin positions.

This procedure was laborious and yielded elevation data only at grid spacings on the order of 1 to 5 centimeters (one-half to 2 inches)—too big to measure roughness at the microtopographic level. Also, the pin would sometimes sink if the soil was soft.

SCOTT BAUER



Technicians Wayne Carstenson (left) and Brent Schroeder use a newly developed laser system to record the roughness of a soil sample. (K-4191-12)

The laser beam, working like an optical pin but without touching the surface, gives the true surface elevation.

Why Measure So Small?

“With water and wind erosion, soil particles being detached and transported have sizes in the order of millimeters,” says Huang. “So to understand these processes, we need to study the surface microtopography resulting from soil grains and aggregates (soil clumps) that move.”

“Water infiltration, soil erodibility, and ease of tillage depend on soil

texture,” says Bradford. “And soil texture is determined by its percentages of clay, sand, and silt.” Clay particles are less than 0.002 mm in diameter, silt is 0.002 to 0.05 mm, and sand is 0.05 to 2.0 mm.

The scanner has the spatial resolution required to detect roughness from millimeter-scale soil particles. It can digitize a 1-meter-long profile in 6 seconds, taking data as close as 0.5 mm (20-thousandths of an inch) apart. For a typical 1-meter-square erosion test plot, it can collect about 1 million elevation points in 50 minutes.



and field studies that quantify how soil roughness affects erosion.

Other ARS researchers also use the scanner. Lawrence J. Hagen, an agricultural engineer at the ARS Wind Erosion Unit at Manhattan, Kansas, uses it to study the processes of wind erosion. "Data collected by the scanner in wind tunnel tests enable us to quantify how soil clods protruding from the soil surface shelter it and prevent erosion," says Hagen. "It also calculates the capacity of the surface roughness to trap and store detached soil particles among the sheltered areas between the soil clods."

Besides studying soil erosion, a team of scientists from the newly established ARS National Soil Tilth Laboratory at Ames, Iowa, uses the scanner to measure water storage on the soil surface from tillage operations and cattle grazing. "Ponding on the surface decreases runoff—and hence erosion—by holding the water in place until it can infiltrate the soil," says soil scientist Jerry Radke.

Entomologist Edwin Berry at the Ames lab also uses the scanner to estimate earth-

worm activity at the surface—which can affect soil tilth. "Some earthworms feed at the surface and move organic material to their burrow openings," says Berry. "They often form a cast near the opening, and the change in size and shape of these casts reflects their activity."

Other USDA agencies also benefit from data collected by the scanner.

The USDA Forest Service Intermountain Research Station at Moscow, Idaho, uses it to measure soil erosion from forest road surfaces, roadside ditches, and wheel ruts from logging trucks, as well as from recently harvested areas. "The major advantage of the laser scanner is that it can measure roughness very accurately," says Edward Burroughs, project leader. "There are other methods to measure surface contours and roughness that are faster and that analyze data more easily, but the measurements are coarser."

By Dvora Aksler Konstant, ARS.

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SCOTT BAUER



Measuring soil surfaces doesn't have to be this arduous. Photographic pin meter operated by technicians Wayne Carstenson (left) and Brent Schroeder will be replaced by the new laser recording system. (K-4192-9)

Bradford and Huang use the scanner to measure surface lowering or rising from two processes: erosion-deposition and wetting-drying. Minute changes such as either of these can be detected only by the scanner.

Erosion-deposition is soil eroding from one place and being deposited somewhere else. Wetting-drying is the process where wet soil swells and dry soil shrinks, which changes the properties of the soil surface.

Since it is portable and can fit into the back of a van or station wagon, the scanner can be used in both laboratory

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Cuphea—Plants With a Beautiful Future

BRUCE FRITZ



Inside a growth chamber, agronomist William Roath examines *Cuphea viscosissima*. (K-4199-1)

The pink, red, and purple-flowered cuphea (coo-FEE-ah) plant may one day add a splash of color to the golden corn tassels and lavender soybean blooms that dot midwestern fields each summer.

ARS scientists hope to domesticate the plant, which grows wild in North, Central, and South America. Some species, members of the *Lythraceae* plant family, have already been released as ornamental ground cover for urban settings in the southeastern United States.

Once modern biotechnology taps the genetic diversity of cuphea and domesticates the plant—admittedly a long-term and high-risk undertaking—farmers should have a ready market for the seed it produces, says William W.

Roath, an ARS agronomist at the North Central Regional Plant Introduction Station, Ames, Iowa.

Roath is curator of a collection of about 400 cuphea strains representing 90 of some 250 species believed to exist. He collected many of the strains in Brazil, working with scientists of that nation's germplasm resources laboratory.

Early this fall, he plans to explore for more species in southern Mexico, along with scientists of Mexico's National Herbarium.

Cuphea seed oil contains large amounts of fatty acids, such as lauric and capric acid, that help give soaps and detergents their cleaning power. Capric acid, a foam-stabilizing component in dessert whips, also shows

promise for expanded use in medical, nutritional, and dietetic applications.

Currently, the U.S. soap and detergent industry gets about half of these fatty acids from petroleum. The other 50 percent comes from coconut and palm kernel oils. In an average year, the 500,000 tons of imported oil cost about \$300 million.

Fluctuations in the price and supply of coconut and palm kernel oils are just one reason for developing cuphea as a U.S. crop, says Roath.

Cuphea oil's high certain fatty acids also makes it preferable to tropical oils. Coconut oil is 45 to 50 percent lauric acid while some strains of cuphea produce an oil that contains nearly 80 percent of the fatty acid.

ARS, the Oregon State University Agricultural Experiment Station, and

member companies of the Soap and Detergent Association are working to develop cuphea's potential as a crop plant. The joint program began in 1984.

The work could pay off in just a couple of decades, instead of the centuries it took to develop other crop plants, Roath says, "because we have the tools of modern science."

Scientists are aiming for a seed production yield of 1 ton per acre. But first, researchers have to tame some of the wild traits that may be detrimental to a cultivated crop. For example, cuphea seed is difficult to harvest because it typically shatters easily from the pod and develops and ripens unevenly over a long time.

Moreover, seed dormancy makes establishing a crop stand unreliable. Few seeds germinate the first year; others may germinate years later.

Overcoming this dormancy has been a painstaking, yet necessary task to quickly grow seed needed for research projects. To do this, Roath removes the seed coat, germinates the seed on wet paper, and transplants the seedlings.

However, Steven J. Knapp, a plant geneticist at Oregon State University in Corvallis, has found a less tedious method to get similar results. He crosses strains of *C. viscosissima* with *C. lanceolata*, a species less prone to dormancy, to get fully fertile hybrids.

Roath believes that *C. viscosissima* holds the most promise for midwestern agriculture because of its adaptation in the wild to midwestern conditions. Interspecific hybridization—crossing species—is one procedure to assist in developing the plant for cropping because, so far, little genetic variation has been found.

Until 1987, the Plant Introduction Station had only three accessions of *C. viscosissima* from Missouri, Virginia, and West Virginia. Then Roath, and horticulturist Mark P. Widrechner at Ames, added 77 more grown from seed

they had collected in a 300-mile-wide area stretching from eastern Kansas to western North Carolina and Virginia.

Chemists Robert Kleiman and Bliss Phillips at the USDA National Center for Agricultural Utilization Research, Peoria, Illinois, helped evaluate the cuphea lines. Although genetic variability is less than researchers had hoped, there were some differences in seed size, yield, and oil content and composition that may one day be exploited to produce crop varieties.

Tests indicate that the *C. viscosissima* lines are rich in caprylic and capric acid, but low in lauric acid—around 4 percent. Both capric and caprylic acids are used in specialized clinical diets to alleviate digestive disorders.

Scientists believe that it may be possible to increase the lauric acid content through mutations. To induce these changes, Knapp is treating plants with chemical mutagens while graduate student Hamadi Ben-Salah at Iowa

State University is searching for the mutations among tissue cultures. ISU graduate research assistant Weiping Chen is working with Roath and ISU biochemist Basil Nikolau to develop biotechnological approaches to tap the genetic diversity of cuphea.

Other hopes for increasing lauric acid lie in crossing *C. viscosissima* with other species.

To help identify plants with desired traits among the accessions of *C. viscosissima* and other species, Chen is searching for enzymes that might serve as genetic markers for those traits.

Traits that seem common among cuphea species include resistance to diseases and insects. But ARS entomologist Richard L. Wilson of the Plant Introduction Station says insect problems may arise when cuphea becomes a widely grown crop. So far, he's seen one possible pest—the larvae of the whitelined sphinx moth—crawling on cuphea plants.

Cuphea growers and landscapers may be pleased to find the plant provides some measure of protection against erosion. At Tifton, Georgia, ARS soil scientist Casimir A. Jaworski has identified selections of *C. llavea* and *C. glutinosa* that overwinter well in the southeastern United States and provide especially good ground cover as spread by rooting of shoots, by underground stolons, and by seeds.

Jaworski and his colleagues have developed four ornamental cuphea plants suitable for the southeast United States. Three of the selections in *C. glutinosa* have been named Lavender Lady, Lavender Lei, and Purple Passion. A single selection of *C. llavea* has been named Georgia Scarlett.—By **Ben Hardin**, ARS.

If you're interested in contacting scientists mentioned in this article, write or telephone the author, Ben Hardin, 1815 North University St., Peoria, IL 61604. Phone (309) 685-4011. ♦

BRUCE FRITZ



(K-4200-1)

DOUG WILSON



Barley harvest in Washington's Palouse Hills. (K-3937-14)

Hoppiness Is Brewing Better Beer

SCOTT BAUER



The refreshing, bitter, yet slightly sweet flavor in a foam-capped glass of beer comes mainly from the extracts of two plants: hops and barley. New varieties of these crops, bred by ARS scientists, have given brewers hops with old-world beer flavor and barley with superior malting qualities.

The newest hop, Liberty, was released this spring by geneticist Alfred Haunold, in Corvallis, Oregon. Liberty boasts the desirable aroma qualities found in its parent, a popular German hop called Hallertauer mittelfrüh. Yet Liberty isn't troubled by the disease problems and poor yields that have all but wiped out plantings of Hallertauer bred in Europe.

Like Mount Hood, a similar hop released in 1989 at the USDA-ARS Forage and Cereal Seed Research Unit by Haunold, Liberty thrives in the temperate Pacific Northwest, producing double the yields of the Old-World variety.

Lush, leafy-green hop vines can grow up to 18 feet or higher, climbing a trellis of wires and poles. Hops are dioecious, which means plants are either male or female. The female plants develop flowers that resemble pale-green miniature pine cones, each of which holds tiny resin glands at the base of each petal. The resins are rich in alpha acids, the chemical compounds that give beer its characteristic bitter flavor.

"Large-scale brewers want a hoppy flavor that blends well with the other flavor components in their beers. Mount Hood and Liberty can fill that role," Haunold says.

The United States is second only to Germany in hop production, growing about 58 million pounds a year, all in Washington, Oregon, and Idaho.

American breweries currently import about 16 million pounds each year, but that amount will likely

decrease when Mount Hood and Liberty became available to brewers in sufficient quantities, says Haunold. The new, European-style hops also hold promise for increased markets overseas.

Six major breweries—three in the United States, two in Canada and one in the Far East—are interested in Mount Hood.

The small but growing microbrewery business, known for its distinct, strongly flavored brews, is another market for new hops. Microbrewery beer typically contains up to five times the hop content of regular beer.

Haunold is currently busy working on several new selections that also stem from the old European hops Tettnanger and Saazer. "Already, local brewers are knocking on my door, asking for samples!" says Haunold.

While hops contribute a quenching, bitter bite, it's the malted barley that gives beer a mellow smoothness. The malting process, which dates back to Egyptian times, starts by steeping barley in water, where it begins to sprout, or germinate. During germination, natural enzymes in the grain convert the starches into sugars. The barley is then dried to make the crunchy malt grains that are sold to brewers.

Good malting barley has a relatively low protein content and yields a high extract, or "wort"—the sugary liquid that comes from mashed malt and water. Those characteristics made Klages barley an industry favorite.

Klages was cooperatively released in 1972 by ARS and the Idaho and Oregon Experiment Stations under the direction of Darrell Wesenberg, of the ARS Small Grains and Potato Germplasm Research Unit.

Today, nearly 20 years later, Klages remains the industry standard to which all other two-rowed malting barleys are compared. (Barley

kernels grow in either two or six rows on each stem.) The ARS Cereal Crops Research Unit in Madison, Wisconsin, along with malting and brewing industry collaborators, played a key role in selecting and testing Klages.

Recent offshoots from the germplasm enhancement and breeding program at Aberdeen, Idaho, include Crystal and Russell. Crystal, a two-rowed barley that resulted from a cross between Klages and the

DAVID FAKONER



Plant geneticist Alfred Haunold takes notes on developing hops. The light green cones take about 4 weeks to reach 1 inch in size. (K-4203-11)

German variety Columba has plumper kernels and sturdier straw—so it isn't easily felled in the field—compared to Klages.

Russell, a six-row barley, also forms strong field stands and has lower protein levels and higher malt extract levels, compared to other commonly planted varieties. Both cultivars have been recommended

for malting and brewing by the American Malting Barley Association, headquartered in Milwaukee, Wisconsin.

About half of the 8.2 million acres of barley planted in the United States are malting varieties. However, notes Wesenberg, "quality malting barley and good feed barley share many favorable characteristics, including disease resistance and high yields. So our breeding work benefits all barley farmers and ranchers who feed barley to their livestock."

Plant breeders will often name their new varieties to reflect the region where the plant was bred, be it a famous historical figure or a geological feature. For instance, Klages was named in honor of the late Karl Klages, former head of the University of Idaho's Agronomy Department. Russell comes from Osborne Russell, a 19th century Rocky Mountain fur trapper who travelled in southern Idaho. Mount Hood is named after Oregon's highest peak, located in the state's northwest region.

Why the name Liberty? "At the time," says Haunold, "things were hopping in Kuwait, and there was a lot of talk about freedom, and one of the brewers I work with suggested Liberty."—By **Julie Corliss**, ARS

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For Vitamin A

Eat an Orange Tomato

The vitamin-conscious may someday want their tomatoes carrot orange instead of catsup red. ARS plant geneticist John R. Stommel is in the process of breeding a super-high-vitamin-A tomato, which is likely to be as orange as a carrot.

Large accumulations of beta-carotene, the precursor that the body converts to vitamin A, usually show up as orange coloring, explains Stommel. Both well-known orange vegetables—carrots and sweetpotatoes—are high in vitamin A.

This new tomato could easily be in the same range of vitamin A content as sweetpotatoes are, ounce for ounce, and about half that of the average carrot, Stommel expects.

To create the high-vitamin-A tomato, Stommel has crossed a commercially cultivated fresh market tomato called Floradade with a wild tomato from the Galapagos Islands, *Lycopersicon cheesmanii*.

Galapagos tomatoes bear clusters of beacon bright orange fruit, each about the size of a pencil eraser. "They're not terribly palatable, very bland and tasteless," says Stommel.

But the tiny fruit are 35 to 40 times higher in vitamin A than current commercial tomato varieties. They contain an average of 58 milligrams per gram of fresh weight of beta carotene compared to about 1.5 milligrams per gram in normal tomatoes.

Since the Galapagos tomato crosses directly with cultivated tomatoes, breeding has been relatively easy, Stommel says.

Fruit from the first generation of crosses is about 1 to 1-1/4 inch in diameter, is bright orange with red overtones, and contains about 30 milligrams of beta carotene per gram of fresh weight.

"Unlike the wild fruit of the Galapagos, which are quite bland, these have a very strong tomato taste, so flavor is not likely to be a problem in the finished version," Stommel says.

This summer, Stommel planted his first crop in field-test plots to see how they will do under natural conditions.

Stommel's tomato won't be the first orange tomato to be seen in the marketplace. A tangerine-colored tomato is available as a specialty product, but it does not have a high vitamin A content. The color comes from a gene not associated with beta carotene.

SCOTT BAUER



Plant geneticist John Stommel inspects fruit from his newly developed hybrid plant. (K-4091-18)

It will probably take about five or six more generations before Stommel will have a tomato ready to present to seed producers.—By **J. Kim Kaplan**, ARS.

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SCOTT BAUER



A high-vitamin-A hybrid (right) is created when pollen from a wild Galapagos tomato is replaced by pollen from a standard tomato. (K-4091-10)



(K-4090-7)

Root Enzymes Are in Control

Plants need iron to live and get it from the soil, through their roots. But in many soils, iron is found in forms that plants can't absorb.

To change it into a form they can take up, roots of some plants acidify the soil, which makes iron more soluble. Then the roots use an enzyme that converts the iron to a form the plant can take up.

While iron is absolutely required for plant survival, too much is toxic and deadly. And too little iron stresses some plants, yellowing the leaves (a condition called chlorosis) and reducing crop yields.

Douglas G. Luster and Marcia J. Holden, plant physiologists, are trying to understand how the root "turns on" an enzyme—called an iron chelate reductase—in response to the stress of iron deficiency. (A chelate binds with metals, such as iron, to make them more available in the soil for plant use.)

Iron is found in two major forms: ferric (Fe^{3+}) and ferrous (Fe^{2+}). Plant roots can only take up the ferrous form. The roots can chemically transfer an electron to ferric iron—an electrochemical process called reduction—transforming it to ferrous iron.

Some plants, such as tomatoes, turn on and off the mechanism for converting iron much better than other plants do.

These iron-efficient plants respond to low levels of soil iron by producing more roots and root hairs that have more of the enzyme needed to make iron available. When there is enough iron of the right form in the soil, the plants don't turn on this mechanism.

Luster and Holden are with the Foreign Disease-Weed Science Research Laboratory in Frederick, Maryland. In studying the characteristics of the proteins that make up this iron chelate reductase enzyme, they have collaborated with Rufus L. Chaney of the Environmental Chemistry Laboratory in Beltsville.

The iron chelate reductase enzyme is located in the plasma membrane, a thin



Plant physiologists Marcia Holden and Douglas Luster inspect tomato plants for iron deficiency. (K-4191-5)

membrane that surrounds root cells. Membranes are composed mostly of fatty components and enzymes, which are made up of proteins.

Holden wants to see how the stress of iron deficiency affects the root's enzyme. "We want to know whether there's actually more of this enzyme being produced in the membrane, or if iron stress merely activates the system that's already there," she says. "A third option is that it's a unique enzyme made only under iron stress."

To study the enzyme, Holden isolates it from the plasma membranes of root cells of tomato plants grown with insufficient iron. She separates the enzyme from the plasma membrane by adding a detergent to separate the proteins from the fat. "The root cell plasma membrane has 'icebergs' of protein floating in a fat layer," she says. "The proteins, including the reductase enzyme, will move into the detergents and become water soluble." This step makes the enzyme easier to purify.

It was Chaney who originally realized that the reduction of ferric to ferrous iron was a necessary step for a plant to take up iron. He theorized that the enzyme must be positioned across the cell-membrane, taking electrons from inside the cell—to change the iron outside the cell to ferrous iron, the form the plant can use.

He found a way to show where the enzyme is active—bathing roots with a stain that shows on reduced (ferrous) iron. In stressed plants—that have been grown with insufficient iron—the roots will turn blue at enzyme sites; in unstressed plants, little dye appears.

Luster and Holden's work with the enzyme in the plasma membrane shows

that the enzyme on the root cell surface is, in fact, an important plant response to iron deficiency. Activity of this enzyme (iron chelate reductase) is a measure of the intensity of a plant's reaction to iron stress.

The scientists measure the enzyme's activity on electrophoretic polyacrylamide gels—a standard laboratory technique that uses a small electric current to separate various proteins into layers within a gel material. Where the enzyme is active, stained "bands" (of a stain to detect ferrous iron) appear on the gel.

"We see more than one enzyme band on the gels," says Luster, "which means that there are several forms of the enzyme, but only some of them are activated when the plant is under iron stress."

So far, they've found that the properties of the enzyme are similar in unstressed and stressed plants. This means that probably the same enzymes are produced by both, but more enzyme is made when the plants are stressed.

Once they identify all parts of the mechanism that responds to iron stress, the important genes—the ones that turn the mechanism "on" and "off"—can be identified. It will then be possible to clone and transfer the gene to plants that are less efficient at responding to iron stress.—By **Dvora Aksler Konstant, ARS.**

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Wheat Tough Enough To Take the Hessian Fly

About one-eighth-inch long, the female Hessian fly emits a sex pheromone from her ovipositor to attract males. (K-4193-13)

ARS researchers were part of the team that dealt the Hessian fly a one-two punch when they zapped the chromosomes of a wheat/rye hybrid with x-rays, creating a new source of Hessian fly resistance for wheat.

First identified on Long Island in 1779, the pest was apparently brought to the United States in the straw bedding of Hessian soldiers fighting in the Revolutionary War. Over time, it has spread to all major wheat growing regions of the United States. Larvae of the insect attack young wheat in the fall and again in the spring, stunting plant growth and causing yield losses of 5 to 10 percent each year.

In early years, winter wheat growers depended on the so-called fly-free date to protect the wheat. They planted their crop after the date in the fall when wheat fields in a given region were generally free of Hessian flies. However, this method was not always reliable. More recently, wheat varieties that genetically resist feeding of the larvae have been used to combat the pest.

But some resistance genes used in varieties have been deployed for more than 10 years and are losing their effectiveness.

"The genetic variability in the fly is such that it can overcome resistance," says ARS entomologist J.H. Hatchett. "To continue to protect

wheat, we have to be able to come up with new genes. As the deployed genes are lost, we have to have new genes ready to replace them."

Hoping to score a knockout, scientists searched for a suitable species that could lend the much-needed resistance to wheat. They selected the rye plant, a distant relative of wheat, because it was known to be highly resistant to the Hessian fly. The scientists are still uncertain exactly what causes the resistance. The young larvae feed on the resistant plants for 2 to 3 days and then die. The phenomenon known as antibiosis appears to cause an incompatible feeding response. "Because rye is a poor host for the pest, we felt

like there was a possibility that in the long run, rye genes may be more durable than those in wheat," Hatchett says. "The trick was figuring out how to transfer the rye gene to wheat chromosomes."

The late Emil E. Sebesta, who was an ARS scientist at Stillwater, Oklahoma, when this study began, had previously used x-rays to move greenbug resistance from rye to wheat.

Based on this research, the scientists opted to use irradiation because of its proven ability to break chromosomes—a necessity to begin the transfer of genes from rye to wheat.

Breaking the chromosomes also eliminates unwanted rye genes, explains Bikram Gill, a Kansas State University geneticist. The research was performed in cooperation with the university's Wheat Genetics Resource Center.

Scientists first crossed resistant Balbo rye and a susceptible common wheat. Chromosomes of the progeny were chemically treated with the compound colchicine, which doubles the number of chromosomes to overcome sterility.

These plants were again crossed with susceptible wheat plants, and the resistant progeny were allowed to self-pollinate. Pollen from the progeny plants was exposed to a low dosage of radiation and then used to fertilize several lines of wheat.

"At this point in the process, it just takes a lot of luck," Hatchett notes. "You hope that the x-rays will break the wheat chromosomes and rye chromosomes and a piece of the rye chromosome carrying the gene for resistance will insert or attach to the wheat chromosome."

Luck was with the researchers. After several generations of testing and selection, pure, resistant sublines were obtained that carried the normal 42 chromosomes of wheat. Using genetic fingerprinting to identify the rye chromatin in the wheat plants, the scientists found three types of translocations: two terminal and one intercalary.

The terminal translocations had a small segment of the rye chromosome attached to the end of a wheat chromosome. The intercalary translocation was formed when a very small piece of rye chromatin was inserted in the middle of a wheat chromosome.

"When a small segment of the rye chromosome is added to the end of a wheat chromosome, a part of the wheat chromosome is usually lost," explains Gill. "But with an intercalary translocation, none of the wheat chromosome is lost, which makes it more valuable than a terminal translocation."

This is the first time an intercalary translocation involving wheat and another species has been found, the geneticist says.

Lines derived from the irradiated pollen were tested for resistance in a greenhouse at Kansas State University. "Resistance was verified by finding dead Hessian fly larvae at the base of the plants," Hatchett says. Germplasm from the three lines will be made available within the coming year to both public and private wheat breeders for use in development of new Hessian-fly-resistant wheat varieties.—
By **Marcie Gerriettes, ARS.**

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Plant geneticist Stan Cox (right) and entomologist J.H. Hatchett check for desirable traits in wheat plants carrying wheat-rye chromosomal translocations. (K-4193-3)

SCOTT BAUER



Technician Ken Oppenlander dissects a Hessian fly-resistant wheat seedling to confirm the presence of dead larvae. (K-4194-6)

Building a Better Strawberry

Since history began, the strawberry has been a favorite fruit. The Roman statesman Cato personally supervised strawberry growing on his estate. And as early as the 1400's, Western European monks were using the wild strawberry to illustrate their hand-written manuscripts.

"Today, strawberries mean good, healthy eating," says Gene J. Galletta, ARS plant geneticist. He and plant pathologist John L. Maas play a key role in ensuring that the supply in the Northeastern United States is plentiful and the quality good.

At the ARS Fruit Laboratory in Beltsville, Maryland, these scientists are breeding disease-resistant plants that produce bigger, better tasting strawberries that are easier for farmers and home gardeners to grow.

Sweet, juicy strawberries not only taste good, but they're also full of nutrition. Low in calories and carbohydrates, the raw fruit is a good source of fiber, potassium, iron, and vitamin C.

And they're a major flavor and/or fruit component of many food items.

Strawberries also contain ellagic acid. According to Maas, "Medical researchers have found, in experimental studies, that this naturally occurring organic compound inhibits the start of cancer caused by certain chemicals."

According to Dr. Gary D. Stoner, of the Medical College of Ohio, the acid "prevents procarcinogens from breaking down and may act as a trapping agent for carcinogenic metabolites."

However, scientists don't yet know how much ellagic acid you would need to eat to reap benefits.

"We know that ellagic acid is found in strawberry roots, leaves, and fruit. In fact, it's also present in several other small fruits and nuts. But we had no idea about the genetics of the acid," explains Maas.

He, Galletta, and Shioy Y. Wang, ARS chemist, have just finished testing about 50 strawberry varieties and

breeding selections to see just how much ellagic acid each has. They found that ellagic acid ranges 4.4 to 9.5 times higher in leaves than in fruit pulp and twice as high as in the seeds. Importantly, this shows that "breeding for high ellagic acid in the fruit should be highly successful."

He estimates that there is about an ounce of ellagic acid in 25 pounds of strawberries.

Maas and Galletta work closely with the North American Strawberry Growers Association in their efforts to

improve the fruit. Scientists from universities in 13 States are also cooperators.

"We're proud of our research program. If all pesticides were banned tomorrow, we'd lose only about 20 percent of the crop to pests and diseases we couldn't control," Galletta says. "Many other crops could be completely wiped out."

Galletta can well be proud; he picked up the strawberry research challenge from George Darrow, who began USDA's strawberry improve-

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Plant geneticist Gene Galletta (left) and plant physiologist John Maas evaluate the yield and quality of a berries from day-neutral plants. (K-4173-2)

ment effort over 70 years ago at Glenn Dale, Maryland. Darrow bred for resistance against red stele root rot, a disease that was devastating to the crop in the 1930's.

Today, Galletta continues to strive for a disease-free plant. Working with plant pathologist Barbara J. Smith and plant geneticist Creighton L. Gupton has brought Galletta a step closer to that goal.

At the ARS Small Fruit Research Station in Poplarville, Mississippi, these scientists have been screening thousands of strawberry plants a year, hoping to find plants not affected by anthracnose, a fungal disease that reached epidemic levels in the 1970's.

"The ARS station here at Poplarville was selected for anthracnose screening because there are no commercial strawberry plantings in the area," Smith says.

"Used alone, none of the fungicides registered for use on strawberries control anthracnose. We've been using a combination of cultural practices and fungicide sprays," Smith says. "But the future lies in resistant varieties." If proper breeding methods are used, anthracnose resistance, according to Gupton, will be easily transmitted from parent plants to offspring.

And now, after about 15 years of work, they will soon be ready to release several strawberry varieties that stand up against this disease. These varieties have been field-tested in Alabama, Louisiana, Florida, North Carolina, and Mississippi.

During field tests, Smith rates the strawberries for yield, fruit size, shape, color, and flavor as well as for resistance to other diseases and insects. "Besides being anthracnose resistant, the selections we're releasing rate high in all these categories," she says.

Strawberries have also been improved in terms of their fruiting timetable. Building on research done by Donald H. Scott and Arlen D. Draper (retired ARS horticulturist and geneticist), Galletta

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John Maas (left) and Gene Galletta examine the growth of micropropagated strawberries. (K-4174-9)

developed two everbearing strawberries, Tribute and Tristar.

These berries, well suited for the Northeast and the Midwest, bear fruit 4 months longer than conventional spring-fruiting types.

A light-insensitive gene from a Rocky Mountain strawberry species was transferred into the new varieties, making them day-neutral.

"Conventional strawberry plants initiate flowers as day length changes in the fall, then bear fruit the following spring. We picked names with 'tri' in them, such as Tribute and Tristar, because they bear fruit in the spring, summer, and fall," he says.

Galletta says that most of the strawberries grown on the East Coast are for local markets and pick-your-own operations. The exception is Florida, the third largest strawberry producer in the United States. California is number one, followed by Oregon. These three states account for 90 percent of U.S. strawberry production.

Galletta, who has spent his entire career trying to build a better strawberry, bites into his work with gusto. "Strawberries appeal to almost everyone," he says. "They're popular in Europe, Asia, Central and South America, and Oceania and are becoming well liked in Africa and other tropical countries. We must work

harder to extend the shelf life of the berries and to develop new berry products that can be shipped without refrigeration. Most importantly, we need to reduce grower costs."

Fumiomi Takeda, plant physiologist at the ARS Kearneysville, West Virginia, research station, believes growers can determine plants' water needs simply by looking for drops of moisture on plant leaves.

"Strawberry plants growing in soil with adequate moisture produce water droplets on leaf margins during the night. But droplets don't form on leaves of water-stressed plants," Takeda says.

"Growers would look for these drops of moisture at dawn and be able to schedule irrigation without having to use expensive moisture-sensing equipment."—By Doris Stanley, ARS.

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Looking Into a Cotton Cell

Imagine being able to study how a plant goes about the process of living while the process is underway instead of having to examine a still life slice of plant tissue.

That's what ARS chemist Judy D. Timpa is doing with cotton by putting to work a machine more commonly seen in medicine than in agriculture.

Timpa and researcher John M. Brown at the University of Missouri Medical School have begun using magnetic resonance imaging (MRI) to look at anatomical and physiological changes that occur during cotton seed and fiber development as the changes actually occur.

To produce its living images, MRI surrounds a subject, in this case a cotton plant complete with roots, plastic pot, and soil, with a powerful magnetic field. So powerful is the field that the nuclei of certain atoms in the plant line up parallel to it.

Then the nuclei are jolted with high frequency radio waves, which causes them to resonate like microscopic tuning forks. Each atom resonates with its own specific faint radio signal, which can be read and translated into a live televised or photographed image.

But the technique does absolutely no harm to tested plants, animals, or people.

"What we finally have is a noninvasive technique that lets us see what is going on inside the cotton plant," Timpa explains. "We've been able to see seed development very clearly."

In a recently completed pilot study testing MRI, Timpa has been able to observe changes in physiology within the cotton boll, including the fiber masses at different stages of development.

A major advantage of MRI is that it doesn't require destruction of the plant.

"I can look at what is going on in the boll where fiber is formed at 8 days after flowering and go back to that same plant to see what is going on at 25

days. It's a whole lot better than relying on the expectation that sibling plants will behave exactly the same way as those sampled on the first date," Timpa says.

Another use Timpa has in mind for MRI is to reveal how cotton plants react physiologically to environmental stresses such as drought as the reaction is going on.

"We can actually track the flow of water molecules through the plant—in the leaves and the bolls—and see what else changes, instead of disturbing the tissue structures in the process of preparing a specimen for analysis or microscopic examination," Timpa says. "With MRI, we've got a whole new horizon for cotton research."—By **J. Kim Kaplan, ARS.**

Judy Timpa is a chemist at the ARS Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124. Phone (504) 286-4275. ♦

Inhibit Melanin: Your Fungal Problems Are Over

The secret to fighting crop-damaging fungi often lies not in destroying them, but in simply disarming them.

So says Michael H. Wheeler, a chemist with the Agricultural Research Service's Cotton Pathology Research Unit at College Station, Texas.

Wheeler says the power behind the fungi's punch is a natural compound called melanin—the same compound that gives fungi their characteristic black or brown color.

Fungi often invade crop plants by forming probes that penetrate plant tissue. In certain fungi, melanin must first accumulate in the cell wall of a specialized structure that grows from the fungus. This structure, called an appressorium, then produces the probes that penetrate the plant tissue.

An ARS team that has included Wheeler, chemist Robert D. Stipanovic, plant pathologist Alois A.

Bell, and others is studying the way that fungi manufacture melanin.

Melanin's importance in fungal attacks has been known for a while. Chemicals used since the 1970's to fight a crop disease called rice blast have operated by blocking production of melanin by the rice blast fungus.

But it was Wheeler and the other ARS researchers who discovered precisely how fungi make melanin. And it was Wheeler who subsequently discovered the same melanin-making process in more than 30 other fungi, including several major culprits causing crop disease.

"Even when melanin isn't essential to a fungal attack on a crop, it may still be crucial to protecting the fungus," says Wheeler. "If you take a fungal structure that has lost its ability to make melanin and put that structure in the soil, there is good evidence that in most cases, it will be broken down by soil microorganisms in a few days or weeks.

"But the ones with melanin can survive in the soil for several years. Also, they're more resistant to ultraviolet and other forms of radiation."

While melanin-inhibiting chemicals such as tricyclazole and pyroquilon are used to prevent rice blast in other countries, notably Japan, they are not registered for use in the United States.

Nor are U.S. chemical companies rushing to register and market those products here because of the high costs of doing so, according to Wheeler.

But understanding exactly how fungi make melanin gives scientists a chance to find other ways to interfere with the process and leave fungi defenseless in the soil, Wheeler says.

"If we can damage the fungus' ability to make melanin, then we could let soil microorganisms or other environmental stresses do the rest," he says.—By **Sandy Miller Hays, ARS.**

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Artificial Flowers, So Lovely, So Lethal to Moths

An insect scientist who fashions fake flowers?

Yes, it's true. But Peter J. Landolt's trick blossom isn't the product of a newfound hobby. It's part of a novel system that annihilates female cabbage looper moths without harming the environment.

The ARS entomologist fashioned a faux flower to resemble a blossom of the tropical night-blooming jessamine shrub, after discovering that female looper moths are attracted to the blossom's scent.

The "petal" part of Landolt's flower is highly reflective white tape, which mimics the shrub's blossom as the sun begins to set (that's moth-feeding time). And, instead of a stamen filled with nectar, Landolt's creation sports a glass capillary tube filled with sugar, synthetic copies of the flower's scents, and an insecticide called methomyl.

The scents were identified and reproduced in the laboratory by chemist Robert R. Heath, who works with Landolt at ARS' Insect Attractants, Behavior, and Basic Biology Research Laboratory in Gainesville, Florida.

Adult females searching for nectar pick up on the scent and follow it to the area. When they are close enough to see the fake blossom, they fly to it and insert their proboscis into the capillary tube, sucking up the deadly mixture.

"Methomyl is a highly toxic chemical," Landolt says. But the beauty of the system is that the poison stays in the tube and in the insect—and doesn't get sprayed into the environment.

In flight tunnel tests—in which adult insects must fly against a wind current to reach the scent and fake flower—the system offered 100 percent control. "Every moth was attracted, fed on our dispenser, and died," Landolt says.



A female cabbage looper moth is lured to a fake flower resembling a blossom of the tropical night-blooming jessamine shrub.

Landolt conducted field tests this past summer and is tabulating results now.

"This promises to be a very effective way to annihilate adult females as they search for nectar," without releasing chemical pesticides into a crop field. By targeting adult females before they lay eggs, a next generation of loopers is thwarted.

Loopers devour cabbage, broccoli, cauliflower, celery, green leafy vegetables, peas, potatoes, tomatoes and other crops—to the tune of at least \$100 million per year.

The research project began as a casual observation by Landolt when he lived in Miami several years ago. Like many Floridians, Landolt had two of the beautiful night-blooming jessamine shrubs in his yard, even though "they stink—so bad they'll give you a headache."

But that's obviously not the effect the shrub has on several kinds of moths. "When the shrubs were blooming, they were always covered with moths feeding on the flowers," he

says. The moths turned out to be two relatives of the cabbage looper.

Heath and Landolt worked to identify which scents were responsible for attracting the moths to the flowers and then reproduced them. Next, they tested the moths' response to different types of sugar solutions and chose the best one. Then they tested many different formulations of the scents, the sugars, and methomyl to pick the best release rate. "We tested pesticide dosages, because we don't want the lure to have so much methomyl that it becomes a repellent. And yet we wanted the best mortality."

Landolt hopes to adapt the system for soybean loopers and perhaps other pests as well.—By **Jessica Morrison Silva**, ARS.

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